SYNTHESIS OF UNNATURAL ENANTIOMER OF NECTRISINE AND ITS BIOLOGICAL ACTIVITY

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Abstract - ent-Nectrisine (2), the unnatural enantiomer of nectrisine (1), a potent α-glucosidase inhibitor, was synthesized from D-glucose derivative. Biological assay showed that 2 had no significant bioactivity.

Introduction
Since nojirimycin (5)1 was isolated as a potent glucosidase inhibitor, many glycosidase inhibitors, which have sugar-like skeleton and nitrogen function located in the ring, were isolated from natural origins.2 Much efforts have also been paid to develop new and efficient glycosidase inhibitors synthetically.3 Nectrisine (1)4,5 was isolated as an immunomodulator from Nectria lucida, and was shown to be a potent inhibitor of α-glucosidase and α-mannosidase. Furthermore, 1 shows inhibitory activity against processing glucosidase, which is involved in glycoprotein synthesis,6 at cellular level as well as enzymatic level.7 While both DAB-I (3),8 reduced form of nectrisine (1), isolated from Arachniodes standishii and Angyloclyx boutiqueanus, and its enantiomer LAB-I (4), chemically synthesized, were reported to have inhibitory activity against glycosidases.9 Moreover, LAB-I (4) inhibits HIV replication more effectively than DAB-I (3), which implies that 4 is a more powerful inhibitor of processing glucosidase than 3.10

![Figure 1](image_url)

Therefore we expected ent-nectrisine (2) to be also an efficient inhibitor of processing glucosidase. Although 2 has been already synthesized as a component of lipopolysaccharides,11 bioactivity of 2 was not known. Thus we were interested in the synthesis and biological activity of 2. We describe herein the synthesis of 2 from D-glucose derivative, and the results of bioassay on inhibitory activity of 2 against α-glucosidases and effects at cellular level.
Results and Discussions

We planned to synthesize 2 from D-glucose, cleaving C5-C6 bond and introducing nitrogen function at C4 by SN2 reaction. For this purpose, multi-hydroxyl functions of glucose had to be protected with different type protecting groups.

We started the synthesis from known 1,2-O-isopropylidene-3-O-benzyl-α-D-glucofuranose (7), which was readily prepared from commercially available diacetone-D-glucose. 1,2-Diol part of 7 was cleaved with NaIO₄, and the resulting aldehyde was reduced with NaBH₄ to give 1,2-O-isopropylidene-3-O-benzyl-α-D-xylofuranose (8) (2 steps, 95%). Hydroxyl group at C5 of 8 was protected as allyl ether (quant.), which is resistant against both acidic and basic conditions. Methanalysis of 9 with acidic ion-exchange resin in methanol gave methyl xylofuranoside (10) (88%) and 2-hydroxyl group of 10 was protected as benzyl ether (86%), similarly as 3-hydroxyl group.

Scheme 1

Scheme 2
Then allyl ether of 11 was deprotected by conventional method\(^3\) (t-BuOK, DMSO, 100°C, 20 min; \(\text{H}^+\); 2 steps, 66%). Acetal linkage was opened by dithioacetal formation (78%). The primary hydroxyl group of the resulting diol (13) was protected as TBDMS ether and secondary hydroxyl group is subsequently mesylated in one pot to give 14 (71%).

Substitution reaction of 14 with azide salts (sodium azide, or tetra-\(n\)-butyrammonium azide\(^{14}\)) in DMF at elevated temperature (90-120°C) did not give the desired product, and attempted reaction to obtain more reactive trifluoromethansulfonate corresponding to 14 only gave decomposed products, presumably because of intramolecular reaction between sulfonate and nucleophilic sulfur atom. Therefore we removed dithioacetal before substitution reaction to form methyl pyranoside (16). Treatment of 14 with \(\text{Hg(CIO}_4\text{)}_2\) in methanol cleaved also TBDMS ether as expected by acid generated during deprotection of dithioacetal, to give dimethyl acetal (15), but not 16. The dimethyl acetal (15) was further treated with HCl in methanol to give methyl pyranoside (16) (2 steps, 78%). Then, mesyl group was substituted with sodium azide in DMF at 120°C to give the azide (17) (91%). Catalytic hydrogenation of azide in the presence of di-\(t\)-butyl dicarbonate (Boc\(_2\)O)\(^{15}\) gave the \(t\)-butyl carbamate (18) (93%), followed by deprotection of benzyl ethers by catalytic hydrogenation to give 19 (79%).

Finally, removal of Boc protecting group and methyl acetal under acidic condition, followed by treatment with basic ion-exchange resin, gave \textit{ent}-nectrisine (2)\(^{16}\) (51%). Specific rotation of synthesized 2 was \([\alpha]_D^{22} -19.4^\circ\) (c=0.49, H\(_2\)O), which had almost the same absolute value and opposite sign compared to that of natural nectrisine (1)\([\alpha]_D^{23} +21.8^\circ\) (c=0.6, H\(_2\)O)).\(^4\)

Synthetic \textit{ent}-nectrisine (2) was assayed for its inhibitory activity against \(\alpha\)-glucosidase (yeast), and processing glucosidase (rat liver microsome). But contrary to our expectation, \(\text{IC}_{50}\) values of 2 against these glucosidases were larger than 1mM. At cellular level, 2 inhibits syncytium formation of Newcastle disease virus-infected BHK cells only above 2.0 mM.\(^{17}\)

In conclusion, we completed the stereospecific synthesis of \textit{ent}-nectrisine (2) in 8.2% of total yield through 15 steps from \(\text{D}\)-glucose derivative (7). Bioassay of the synthetic \textit{ent}-nectrisine (2) revealed that it shows extremely low inhibitory activity against glucosidases at enzymatic and cellular level. These results indicate that the relationship of natural nectrisine (1) and \textit{ent}-nectrisine (2) is not parallel to that of DAB-1 (3) and LAB-1 (4). Further study of structure-activity relationship of nectrisine-type analogs should be required and are in progress in this group.\(^{18}\) Detailed results will be reported in a full account.

**REFERENCES AND NOTES**


16. Spectroscopic data of synthetic 2: $^1$H-NMR (300 MHz, D$_2$O) $\delta$ 3.08-4.15 (5H, m), 7.70 (1H, s); $^{13}$C-NMR (75 MHz, D$_2$O) $\delta$ 61.7, 77.3, 78.7, 83.8, 170.9; IR (KBr) 3270, 2920, 1640, 1560, 1400, 1050, 870 cm$^{-1}$.

17. Methods of the bioassay were the same as described in reference 7.


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